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RH: Estimating Maryland's Black Bear Population. Bittner et al.

Estimating Population Size of Maryland's Black Bears using Hair Snaring and DNA Analysis

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Abstract: Black bear (Ursus americanus) populations have expanded in Maryland since the late 1970s. Previous attempts to estimate bear numbers have been hampered by access to private land and manpower shortages. The development of hair snaring techniques, coupled with genetic fingerprinting, provides a more efficient technique than traditional mark-recapture methods to estimate black bear numbers in western Maryland. In May-June 2000, we established 108 grids throughout occupied bear range in Garrett and western Allegany counties in western Maryland. We established hair traps in each grid for 4 week-long sampling periods. Hair samples that were snagged on barbed wire were collected after each sampling period and kept for DNA analysis. We subjected 330 hair samples to DNA analysis, and identified 92

individual bears. We identified 45 males and 43 females, and the gender of an additional 4 bears could not be determined. We used Program CAPTURE to estimate the bear population in western Maryland, and a total of 227 bears (95% C.I. 166-337) were estimated to occupy the 2,152 km² area, 10.5 bears/100km^2 (95% C.I. 7.7-15.7). We found this technique to be more practical for estimating bear numbers in western Maryland than the traditional mark-recapture technique of running trap lines. Costs were substantially less per bear marked in 2000 than previous attempts.

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Black bears were found across Maryland when the state was settled in the 1600s. Like most other states in the eastern US, black bears in Maryland became rare or were extirpated as areas were colonized in the 1800s. In some counties in Maryland, bounties were established to encourage people to eradicate bears (Garner and Mathews 1992a). By 1850, black bears remained only in the remote mountains of western Maryland. Paradiso (1969) stated that by 1956, only 12 bears were believed to be in the very remote areas of Garrett and Allegany counties. Only in the last 25 years have black bears become more common in western Maryland.

A key component of Maryland's bear management program is determining dynamics of the black bear population in western Maryland. In 1991, a mark-recapture study was conducted to determine bear population size in Garrett County. At that time, most of Maryland's bears were located in this westernmost county of the state. Results of that study indicated that there were 79-167 bears in Garrett County (Garner and Mathews 1992b).

The application of genetics to wildlife, and to bear research in particular, has increased the opportunity to manage bear populations (Paetkau and Strobeck 1998, Woods 1998, Waits

1999). Foran et al. (1997) describe DNA analysis of hair samples collected using glue patches. The use of barbed wire to collect hair samples from bears in eastern North America has been conducted in Arkansas, Florida, Georgia, Kentucky, Louisiana, New Jersey, North Carolina, Ontario and Virginia (Clark and Dobey 2001).

Population size of animals with large home ranges, including bears, is difficult to estimate with mark-recapture techniques (Garshelis 1992). However, in recent years, mark-recapture estimates using DNA fingerprinting have been developed for black and grizzly bears (U. arctos) (Boulanger 1998, Woods et al. 1999, Mowat and Strobeck 2000, Poole et al. 2001). The objective of this study was to utilize these techniques to estimate the population size of black bears in western Maryland.

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METHODS

Study Area

The study area covered approximately 2,152 km² of the western edge of the Ridge and Valley Province in western Allegany County, and the Appalachian Plateau Province in Garrett

County. Land cover types consisted of oak-hickory and northern hardwoods forests. Agricultural land was interspersed throughout the study area. The area was bordered by Pennsylvania to the north, and West Virginia to the south and west. The North Branch of the Potomac River also provided the border to the south, but was not considered a constraint to bear movement in that area. Although black bears were occasionally found in other areas of Maryland, the study area constitutes the core of occupied bear range in Maryland.

Field Techniques

We based study area selection on a number of factors, including estimated bear densities, accessibility, available manpower, cost and cell size. We divided the study area into 108 square grids, with each grid approximately 19.9 km². Grid size was based on the smallest home range of female bears with cubs (Mowat and Strobeck 2000). Dateo (1997) found female Maryland bears with cubs to have an average spring-summer home range of 26.7 km². Ideally, grid sizes less than ½ the spring-summer home range should have been used, but manpower shortages precluded using that many grids.

The use of hair traps to collect hair samples is a relatively new technique. There has been much variation in establishing hair traps (D.L. Garshelis pers. commun.). Some researchers have used hanging baits (Mowat and Strobeck 2000), while others have used liquid bait poured within the bait station (Woods et al. 1999). Others recommend moving hair traps between periods, while some recommend leaving the bait station where it is, but changing the type of bait between periods (Boulanger 1998, D.L. Garshelis pers. commun., G. Mowat pers. commun.). In our study, we decided to leave the hair traps at 1 location and use the same bait type for each period.

Four 2-man crews subjectively placed a hair trap to maximize visitation by black bears within each grid throughout western Maryland (Fig. 1). Each hair trap consisted of 1.89 liters of

molasses poured on a stump or tree trunk, which was then surrounded by a single strand of barbed wire approximately 50 cm above the ground. Some cubs may have been too small to be sampled by a 50 cm high wire, but we felt that a lower wire would not sample some larger bears because they may step over the wire. On average, a 2-man crew could place 7-8 hair traps during an 8-hour workday.

We established all hair traps during the week of 22 May 2000. Hair traps were checked 7 days after establishment. A white piece of paper was passed behind each barb to assist in determining if hair was present on the barb. We removed each hair sample (all hair on one barb), whether bear or another animal, from the barb and placed it in a # 3 coin envelope. Field staff did not wear latex gloves during the collection period, as gender contamination with hair samples was not considered relevant (T.L. King, pers. commun., O. Bres, pers. commun.). Each envelope was then uniquely numbered and placed in a larger brown kraft envelope. We filled a # 3 coin envelope with silica beads and placed it in each of the larger brown kraft envelopes to control moisture. All brown kraft envelopes were then placed in a freezer until relayed to the USGS Aquatic Ecological Laboratory, Kearneysville, WV. Personnel at the laboratory identified and removed all black bear hair samples from the total sample set.

Once all hair samples were removed from the barbed wire, hair residue was burnt from the barb using a butane lighter. Each hair trap was then rebaited with the same amount of molasses. Sampling was conducted for 4 7-day survey periods. We completed all surveys by 23 June 2000.

Molecular Genetic Analysis

DNA was extracted from the bear hair follicles using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA). Microsatellite DNA amplification was performed in 2 stages. First

Stage analysis consisted of the amplification of 7 microsatellite DNA loci using the PCR primers described in Paetkau and Strobeck (1994) and Paetkau et al. (1995). We did not use an eighth locus (Locus G10P) because it failed to amplify consistently in all samples. Samples determined to have identical genotypes were subjected to Second Stage analysis of 4 additional microsatellite loci (Taberlet et al. 1997, Paetkau et al. 1998) to increase the probability that 2 samples were in fact taken from the same individual and not simply reflective of direct relatedness (e.g., siblings or half-siblings). Two or more distinct samples exhibiting identical multilocus genotypes upon comparison of 12 microsatellite loci were in all likelihood obtained from the same individual (indicative of recapture). Sex identification was performed via the PCR using male specific (Y-chromosome) primers described by Taberlet et al. (1993).

Statistical Analysis

We analysed the multilocus genotype generated for each individual from the series of PCR amplifications to determine uniqueness of each hair sample. We calculated estimates of individual pair-wise genetic distances, using the proportion of shared alleles algorithm, using a 32-bit version of Microsat 1.5d (Eric Minch, Stanford University). Pair-wise genetic distances of zero were indicative of identical multilocus genotypes.

We estimated the black bear population size using mark-recapture models in Program CAPTURE (White et al. 1982). We selected a model based on the model selection tests performed by CAPTURE (Otis et al. 1978), simulation results from other studies (Mowat and Strobeck 2000) and our knowledge of bear behavior.

RESULTS

We determined that 330 of 1,200 hair samples were from black bears. Most samples that contained 5 or more bear hairs could be amplified (89.3%, 184/206), while only 22.6% (28/124)

of samples that contained fewer than 5 hairs could be amplified. We amplified 212 hair samples with 7 microsatellite loci; 4 showed signs of contamination (hair from more than 1 bear), and 114 yielded poor amplification (not enough DNA material to identify an individual bear). Eighty of the 114 samples (70.2%) with poor amplification came from hair traps where other hair samples were amplified during the same survey period. The remaining 34 samples (29.8%) with poor amplification were the only hair samples collected at a hair trap during a given survey period.

From the 212 amplified samples, 92 unique bears were identified. Of 92 bears sampled, 73 were sampled at 1 trap station on a single occasion and 19 were sampled at more than 1 trap station either during the same survey period or multiple survey periods. Bears that were sampled at different hair traps during the same survey period were not considered recaptured. We identified 45 males, 43 females and 4 bears of unknown gender during the gender determination test.

Of the 19 bears sampled at more than one station, 6 were females and 13 were males. Two of the females were sampled in 3 different survey periods, all the others were sampled twice. All the females were recaptured at the hair trap where originally captured. Of the 13 multi-sampled males, 1 was sampled during 2 survey periods, but at 4 different hair traps; 1 was sampled at the same hair trap during 3 survey periods; and the remainder were sampled twice. Three of the males were recaptured only at the hair trap where originally captured, all others were recaptured at different hair traps.

We collected black bear hair samples at 60.2% (65/108) of the hair traps, with hair from 64.6% (42/65) of these hair traps providing enough genetic material to identify individual bears. We identified 92 bears at these 42 hair traps, 2.19 bears/hair trap. Twenty-two hair traps

collected hair from 1 bear, 11 hair traps from 2 bears, 2 hair traps from 3 bears, 2 hair traps from 4 bears, 1 hair trap from 5 bears, 3 hair traps from 6 bears, and 1 hair trap from 11 bears. The number of new captures varied between survey weeks, as did recaptures (Table 1).

Although we know bears move between the 3 states (MD, PA, WV), we initiated this study prior to the breeding season. The survey ended at what we believed to be the beginning of the breeding season, and we did not notice a substantial increase in new captures during the third and fourth survey weeks. Only 1 bear was known to have died in the study area during this survey (H.A. Spiker, pers. commun.), and no births were believed to have occurred. Thus, we believe lack of demographic closure was not a major bias to this study.

Model selection indicated that model M_o was the appropriate model. Model M_o assumes equal catchability within and among sessions, an unlikely outcome in wild populations (Pollock et. al. 1990). We discarded this model because we believed that capture probabilities were not equal in our study. We chose model M_t over model M_h because there were few recaptures, especially females. Otis et al. (1978) stated that model M_h was considered good and robust if trapping was performed on a large number of occasions and number of recaptures was substantial on each occasion. Although recapture rates were higher in males, and heterogeneity was detected in males, we determined that it would be more appropriate to combine male and female data sets to estimate population size (G. Mowat, pers. commun.). We also had 4 bears where gender could not be determined, which would have been discarded if we used only the male and female data sets. Although population estimates varied by 15% between the 3 models, we selected model M_t because our sample size was low and we captured more bears during the second survey period than the first, an indication of time variation (White et al. 1982).

Using model M_t, the bear population was estimated to be 227 bears, with a 95% confidence interval of 166 – 337 (Table 2). We calculated bear densities in our study area to be 10.5 bears/100 km^2. This included total landmass in the study area. We suggest that bear densities were greater in Garrett County than western Allegany County. Only 6.5% (6/92) of the bears were identified from hair traps in Allegany County, yet this county contained 20.4% (22/108) of the hair traps.

DISCUSSION

The recent development of using genetic fingerprinting to estimate size of wildlife populations has provided bear biologists with a more efficient tool to estimate bear numbers than traditional mark-recapture techniques. The technique was pioneered with grizzly bears and black bears in British Columbia (Woods et al. 1999). It has applicability to estimating black bear population size in Maryland, because bears are primarily found in a 2 county area in the western part of the state.

We estimated a black bear population of 227 bears in western Maryland from the Cumberland area in western Allegany County to the West Virginia state line. The quantity and quality of bear habitat was determined to be greatest in western Maryland (Rasberry and McCorkle 2002), and we believed bear densities were higher in Garrett County than Allegany County. Although Program CAPTURE found lack of evidence to determine violation of closure, male recaptures indicated that there was movement of males between grids. We also knew that bears move across state boundaries. Maryland tagged bears have been recovered in Pennsylvania and West Virginia, and bears tagged in those states have been recovered in Maryland (Bittner 1997). However, immigration and emigration were believed to be equivalent, with no net movement in or out of the study area.

The black bear population appears to have increased in density from 7.4 bears/100km² (95% C.I.: 4.6-9.9 bears/100km²) in Garrett County (Garner and Mathews 1992b) in 1991 to 10.5 bears/100km² (95% C.I.: 7.7-15.7 bears/100km²) in western Maryland in 2000. The increased density in 2000 occurred despite the inclusion of western Allegany County, an area of relatively low bear density. Sardine bait station surveys conducted annually in both counties indicated a higher visitation rate in Garrett County than Allegany County (5 year average of 33.0% vs. 7.2%, respectively) (Spiker 2002).

Maryland's density was low compared to black bear densities reported by Garshelis (1994). He summarized black bear densities determined by mark-recapture studies across black bear range. He reported densities for 6 study areas in the southeast, ranging from 8 bears/100km² in Arkansas to 86 bears/100km² in Virginia. Only 2 of the study sites in the southeast reported densities less than ours. Recently, Willey et. al. (1996) found 31-34 bears/100km² in the northwestern Appalachian Mountain region of South Carolina, while Bowman et. al. (1996) reported that 48-63 bears/100km² were found in the White River National Wildlife Refuge in Arkansas, a coastal plain population.

Comparison of bear densities between Pennsylvania, West Virginia and Maryland is difficult because of the various data collection methods used in each state. Pennsylvania and West Virginia are able to collect information from harvested bears because of their bear hunting seasons. In Pennsylvania, tagged bears that are killed by hunters are the recapture component of their population estimate. Similar techniques are used in portions of West Virginia, but not near the Maryland border. Black bear densities in the 3 Pennsylvania counties just to the north of Garrett and Allegany counties were estimated to be 8.4/100km² in 2000 (M. Ternent, pers. commun.). This estimate included total landmass in these counties, and was slightly lower than

that found in Maryland. In West Virginia, population estimates were not available for counties bordering Maryland. The 3-year average for mortality data in the 4 West Virginia counties that border Maryland showed average total bear mortality at 6.3 bears/100km² in 2000 (W. Igo, pers. commun.).

In Maryland, black bears have been classified as a forest game mammal with a closed hunting season. Thus, the ability to use hunter-harvested bears for population reconstruction in Maryland is nonexistent. The use of genetic fingerprinting on hair samples collected at snagging stations provided an efficient method to estimate black bear numbers in Maryland. This technique is especially well-suited to the relatively small area of occupied range in Maryland.

MANAGEMENT IMPLICATIONS

We plan to use this technique at 5-year intervals to estimate bear numbers in Maryland. We currently utilize an annual summer sardine bait station survey to track bear population trends in western Maryland, and feel that it is unnecessary to estimate bear numbers more frequently than 5 year intervals. Our first population estimate was conducted in 1991, and this survey was conducted in 2000. It verified what many believed, that Maryland's bear population has increased in the core bear area of the state.

We feel that genetic fingerprinting and the hair snaring technique were appropriate techniques for us. Our bears are restricted to a fairly small part of the state, and these techniques provided the ability to survey a larger area in a shorter time frame than the traditional mark-recapture study where bears are trapped, tagged and released. Although it was labor intensive for a short period of time (five weeks), manpower requirements were far less than traditional mark-recapture techniques.

Our study area was easily accessible and field crews established hair traps more efficiently than originally planned. We wanted all traps placed in a 1-week period, and established 4 crews plus several other individuals to set all 108 hair traps. We believed a crew could establish 4-5 hair traps/day. However, it became apparent that a crew, on average, could place 7-8 traps per day. Thus a crew could place 35-40 stations in a 5-day workweek. In the future, we will survey the same area, reduce the grid size, and increase the number of stations that a crew will be required to establish. We believe we can establish at least 140 stations in the same 2,152km^2.

Our total study cost was $30,545, or $332/marked bear. This is substantially less than the cost per trapped bear in the 1991 study. In that mark-recapture effort, 19 bears were captured during 7 trapping periods. Bears had been marked in previous years by field staff and were part of the recapture component of that study, but there are no feasible methods to calculate costs for the pre-1991 trapping efforts. The total cost for the 1991 trapping project was $11,861, or $624/marked bear.

In our study, we collected all hair samples from each barb. We did not discard hair samples from non-target species, as we left this decision to the USGS laboratory. However, in the future we will screen the samples on site, and discard any samples that are not comprised of black hair. Even though some white chest blaze bear hair may be discarded, we don't believe that will be significant. Presorting hair samples will reduce the workload for the laboratory, resulting in reduced costs and quicker analysis.

We recommend that all bear hair samples be submitted for DNA amplification. In our study, only 23.4% of samples with less than 5 hairs could be amplified. However, that provided

an additional 29 samples (13.7% of the total samples). We believe this is a significant amount, and encourage others to test all bear hair samples.

We don't believe that the number of poorly amplified samples significantly biased the results of this study. Seventy percent of the poorly amplified samples came from hair traps where other bears were identified during the same survey period. We believe that most, if not all of these samples, were from these identified bears.

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Table 1. Captures and recaptures of black bears based on genetic fingerprinting in western Maryland, 2000.

Survey period	Total bear captures	New captures	Recaptures
22 – 28 May	Establishment week	Establishment week	Establishment week
29 May – 4 Jun	21	21	0
5 – 11 Jun	35	32	3
12 – 18 Jun	31	26	5[a]
19 – 23 Jun	23	13	10[b]
Total	110	92	18

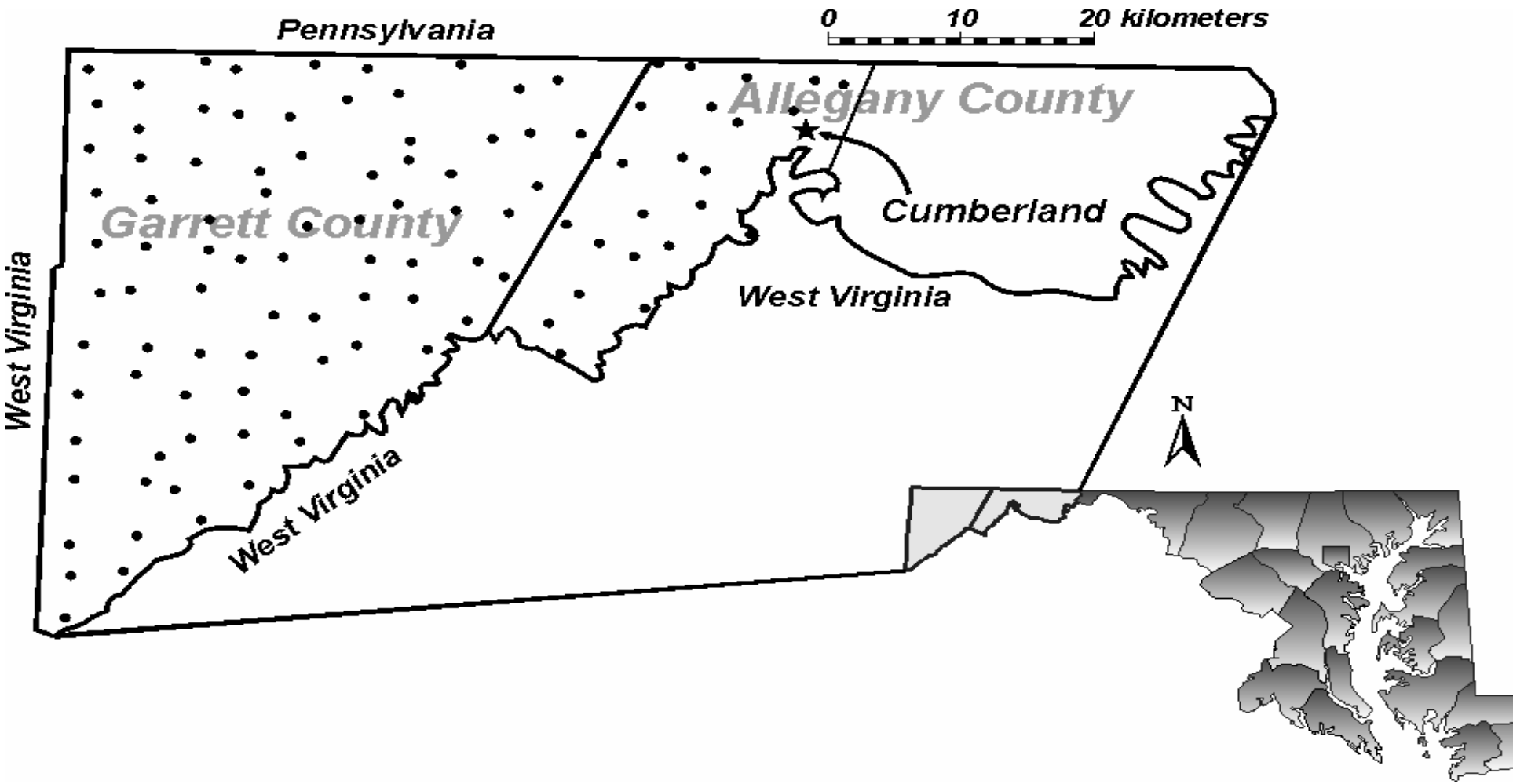
[a] Includes 3 bears from 29 May – 4 Jun and 2 bears from 5 – 11 Jun.

[b] Includes 4 bears from 29 May – 4 Jun, 3 bears from 5 – 11 Jun and 3 bears from 19 – 23 Jun.

Table 2. Population estimates in Program CAPTURE for the western Maryland black bear population from DNA analysis of hair collected at hair trap sites during spring-summer, 2000.

Model	N	SE	95% C.I.
M_o-Null	230	43.6	168-344
M_h-Jackknife	200	16.9	172-238
M_h-Chao	339	92.2	214-593
M_b-Zippin	205	96.2	119-572
M_{bh}-Removal	116	16.4	100-172
M_t-Darroch	227	42.2	166-337
M_t-Chao	258	61.0	175-425
M_{th}-Chao	323	101	194-616
M_{tb}-Burnham	113	20.7	97-197
$M_{pollock/otto}$	131	12.5	114-163

Figure 1. Study area boundary and hair snagging locations (dots) during the spring-summer 2000 in western Maryland.


Pennsylvania
0 10 20 kilometers
Allegany County
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Cumberland
West Virginia
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